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Agents in the Treatment of Progressive Breast Cancer

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13. Abstract (Maximum 200 Words) (*abstract should contain no proprietary or confidential information*): Our overall objectives are to evaluate the antitumor effects and biological activities of Type 1 and Type 2 effector T cells in local and disseminated breast cancer. To date, we have developed and characterized in part, a murine breast cancer model that correlates with defined breast cancer classification stages of clinically progressive disease. Using the TSA adenocarcinoma, we assessed the phenotype and kinetics of tumor infiltrating lymphocytes (TIL) in mice challenged sc in the mammary region. At various times, tumors were extirpated and cell suspensions were analyzed using flow cytometric techniques. We show that T cells infiltrate the tumor site by day 7 following tumor challenge. Both CD4 and CD8 TILs were predominantly CD44^{High} and expressed CD25, CD69, CD95 and CD122 activation markers. Cytokine releasing profiles, as detected by intracellular cytokine staining, showed that activated CD4/CD44^{High} and CD8/CD44^{High} TILs produced elevated levels of IL-4, IL-10, GM-CSF and TNF suggesting that type 2 immune responses were elicited. Activated CD4/CD44^{High} TIL numbers and frequencies reached peak levels at day 21 that markedly decreased by day 28 following tumor challenge. In contrast, activated CD8/CD44^{High} TIL numbers were detectable by day 14 however at substantially lower levels and with slower kinetics than that of corresponding activated CD4/CD44^{High} TIL subpopulations. Over time (>21 days), numbers of NKT cells (DX5⁺CD3⁺) producing IFN- γ , IL-4, IL-10 and GM-CSF were markedly elevated, suggesting a recruitment of NKT cells at later stages of tumor progression. Concomitantly, elevated levels of IP-10, TGF- β and Macrophage Inhibitory Protein (MIP) were detectable and correlated with a continued progression in tumor growth and metastases. Further characterization of effector T cells and the chemokine/cytokine environment during progressive malignancy will aid in understanding the underlying mechanisms of the efferent phase of the antitumor response and provide insight for development of more effective therapeutics to breast cancer.

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INTRODUCTION

Adoptive CD8 T cell immunotherapy has been shown to be a viable modality for the treatment of certain human cancers. Aside from their direct cytolytic potential, CD8 T cells can be further classified into two distinct effector cell types based on their cytokine-secreting profiles following tumor antigen encounter. Type 1 CD8 T cells (Tc1) produce IFN- γ whereas, Type 2 CD8 T cells (Tc2) secrete IL-4, IL-5, IL-10 and GM-CSF. Such cytokines can not only have diverse inhibitory effects on tumor cells themselves, but also affect the nature of the immune response toward progressively growing malignancy. Although the existence of Tc1 and Tc2 effector cells have been demonstrated in patients with various clinical conditions, the nature and regulatory roles of these T cell subpopulations in tumor immunity and immunotherapy remain unclear. It is conceivable that these "multifunctional" subpopulations can effectively regulate breast tumor growth and dissemination by different mechanisms. More importantly, since many breast cancers demonstrate considerable heterogeneity in the clinical course of their disease, treatment with tumor-specific immunotherapeutic agents that potentially afford multiple and diverse mechanisms of tumor eradication may enhance therapeutic benefits and provide a more favorable clinical outcome. Moreover, it is our contention that immunotherapy with tumor reactive Tc1 or Tc2 cells can initiate host-mediated antitumor responses and thus act as potential cellular vaccines that can not only act as primary therapeutic agents but also be effective as adjuvant therapies in patients that do not tolerate conventional clinical treatments.

BODY

The original Aims of the program after its first year remain unchanged. In **Aim 1**, we will develop and characterize a murine breast cancer model that correlates with defined TNM breast cancer classification stages of clinically progressive disease. This model will utilize an aggressive mammary adenocarcinoma cell line, expressing a surrogate tumor antigen (HA), that will be evaluated for three defined clinical stages based on anatomic tumor measurement and dissemination. In **Aim 2**, we will assess the immune responses of either untreated or treated tumor-bearing mice receiving adoptively transferred tumor antigen-specific Tc1 or Tc2 effector cell subpopulations at various stages of disease progression. In general, these studies will assess (i) can adoptive immunotherapy with tumor-antigen specific Tc1 and Tc2 effector cell subpopulations be an effective therapeutic agent in breast cancer and (ii) provide insight on their potential to initiate and/or influence effective recipient-mediated anti-tumor immune responses in individuals with either localized or disseminated malignancy.

A number of the experiments proposed in **Aim 1** have been achieved or are near completion. To initially address potential host immune responses among untreated mice, we developed and characterized an orthotopic mammary tumor model using the TS/A adenocarcinoma. We have shown that these tumors progressively grow in vivo without evidence of spontaneous regression when injected subcutaneously ($1-5 \times 10^5$ cells) into the mammary fat pad. Moreover, these tumor cells undergo spontaneous metastases to lymph nodes, lung and liver that is grossly evident by days 18-25 following tumor challenge. Subsequently, this provides a model that correlates, in part, with both local and clinically disseminated stages of progressive breast cancer. Upon investigation of local immune responses we show that T cells infiltrate the site of tumor growth by day seven following tumor challenge. B cell numbers were negligible at the site of tumor growth. Both CD4 and CD8 tumor infiltrating lymphocytes (TIL) were predominantly CD44^{High} and expressed CD25, CD69, CD95 and CD122 activation markers. Moreover, cytokine releasing profiles, as detected at the single cell level by intracellular cytokine staining and flow cytometry, showed that activated CD4/CD44^{High} and CD8/CD44^{High} TIL cells produced elevated levels of IL-4, IL-10, GM-CSF and TNF suggesting that type 2 immune responses were elicited. Activated CD4/CD44^{High} TIL subpopulations were detectable by day 7 with peak levels at day 21 following tumor challenge.

Whereas, at later stages of tumor progression, activated CD4/CD44^{High} TIL cell numbers and frequencies substantially decreased with time (>28 days). In contrast, activated CD8/CD44^{High} TIL cell numbers were detectable by day 14 post-tumor challenge and appeared to steadily increase with time. However, at substantially lower levels and with slower kinetics than that of corresponding activated CD4/CD44^{High} TIL subpopulations. Over time (>21 days post tumor challenge), the numbers of NKT cells (DX5⁺CD3⁺) producing IFN- γ , IL-4, IL-10 and GM-CSF were markedly elevated, suggesting a recruitment of NKT cells at later stages of tumor progression. Concomitantly, elevated levels of IP-10, TGF- β and Macrophage Inhibitory Protein (MIP) were detectable and correlated with a continued progression in tumor growth and metastases. Collectively, we suggest that recruitment and presence of "regulatory" cells and/or select immunoregulatory factors (i.e. chemokines/cytokines) within the tumor environment at select stages of tumor maturation may aid, in part, in regulating effective host antitumor responses that result in aggressive malignancy. Further experiments will be performed to assess host immune responses at sites distal to primary tumor growth and at later time points following tumor challenge in an effort to characterize immune responses at different stages of metastases.

In parallel studies, we are assessing the role of tumor antigen-specific Tc1 and Tc2 effector T cell subpopulations as potential therapeutic agents and investigating their direct and indirect affects on the immune response in animals with either localized or disseminated breast cancer malignancies. Currently, we are developing to near completion, a TS/A tumor cell line expressing a surrogate tumor antigen. Briefly, TSA mammary adenocarcinoma cells will be transfected with influenza hemagglutinin (HA) cDNA. These transfected mammary tumor cell lines, expressing the surrogate HA antigen, will be injected either sc or iv into syngeneic recipient mice to induce a "local-regional" or "disseminated" mammary tumor disease state, respectively. At different time intervals following tumor challenge, graded numbers of polarized populations of HA antigen-specific Tc1 or Tc2 CD8 cells, generated from HA-TCR transgenic mice, will be adoptively transferred and therapeutic efficacy will be evaluated by tumor growth rate and survival. To date, we have successfully generated and characterized cytokine polarized HA antigen-specific Tc1 and Tc2 cytolytic effector T cell subpopulations derived from HNT.BALB/c (H2-K^d) transgenic mice that contain the same T cell receptor specific for HA antigen presented by the MHC Class I H2-K^d molecule. Phenotypic and functional assessment of freshly generated HA antigen-specific effector cells has been completed in vitro using flow cytometric, functional and mRNase protection assays. We show that both Tc1 and Tc2 effector T cell populations were found to be highly cytolytic to HA peptide pulsed tumors in vitro. The cell surface phenotype of both effector cell populations was determined to be CD8⁺, CD4⁻, CD62L^{low}, CD44^{High}, CD25⁺, and Ly6C⁻ (typical effector cell phenotypes). Tc1 secreted IFN- γ upon restimulation in vitro, whereas Tc2 generated large amounts of IL-4, IL-5, IL-10 and GM-CSF. After experiencing some initial problems with reagents obtained earlier, TS/A tumor cells transfected with HA (TS/A-HA) are currently being drug-selected (G418) and cloned. We anticipate that TS/A-HA tumor cells lines will be available for in vivo studies by the end of September 2002. Once this is completed, we will resume our studies pretty much as outlined in the original application. We will begin next with the experiments looking into the therapeutic effects of adoptively transferred HA tumor antigen-specific effector cell subpopulations and their potential affects and/or influences on tumor-bearing recipient immune responses to progressive tumor growth following treatment.

KEY RESEARCH ACCOMPLISHMENTS

- A. Development and characterization of a murine breast cancer model that correlates with defined TNM breast cancer classification stages of clinically progressive disease.

- TS/A mammary tumor cells express high levels of surface MHC Class I and CD44, but not MHC Class II, CD95 or FasL in vitro.
- TS/A mammary tumor cells progressively grow in vivo without evidence of spontaneous regression when injected orthotopically into the mammary fat pads.
- Orthotopically injected TS/A mammary tumor cells undergo spontaneous metastases to lymph nodes and systemic organs that is grossly evident between days 18 and 24 post tumor challenge.
- Development of a mammary tumor model to investigate tumor antigen-specific T cell subpopulation responses: (i) the generation and characterization of HA antigen-specific Tc1 and Tc2 CD8 Effector T cells from HA-TcR transgenic mice (*task accomplished*) and (ii) generation of a TS/A tumor cell line transfected with influenza hemagglutinin (HA) cDNA (*task near completion*).

B. Assessment of immune responses in TS/A mammary tumor-bearing mice at various stages of disease progression.

- T cells infiltrate the site of tumor growth by day 7 following TS/A tumor challenge.
- Tumor Infiltrating Lymphocytes (TIL) contain elevated cell numbers and frequencies of IL-4, IL-10, GM-CSF and TNF-producing CD4 and CD8 T cells that increase with tumor progression suggesting that Type 2 immune responses are elicited.
- “Activated” CD4/CD44^{High} TILs are detectable by day 7 with peak levels at day 21 following tumor challenge. However, at later stages of tumor progression, cell numbers and frequencies substantially decrease with time (>28 days).
- “Activated” CD8/CD44^{High} TIL cell numbers are detectable by day 14 following tumor challenge and increase with tumor progression. However, at substantially lower levels and with slower kinetics than that of corresponding “activated” CD4 T cell subpopulations.
- High proportions of both “activated” CD4/CD44^{High} and CD8/CD44^{High} TIL subpopulations co-express either CD25, CD122, CD95, or CD69 activation markers.
- Elevated levels of NKT cell subpopulations (DX5/CD3) producing IFN- γ , IL-4, GM-CSF, IL-10 and TNF are present at the site of tumor growth by day 21 following tumor challenge, suggesting a recruitment of NKT cells at later stages of tumor progression.
- Elevated gene expression levels of the immunoregulatory cytokines Macrophage Inhibitory Factor (MIF), TGF- β 1 and TGF- β 3 are detectable in progressively growing tumors.
- Elevated gene expression levels of the anti-angiogenic/chemoattractant chemokine IP-10 are detectable in tumors with peak levels occurring at early time points following tumor challenge (Days 4 to 7).
- Collectively, we suggest that recruitment and presence of “regulatory” cells and/or select immunoregulatory factors (i.e. chemokines/cytokines) within the tumor environment at select

stages of tumor maturation may aid, in part, in regulating effective host antitumor responses that result in aggressive malignancy.

REPORTABLE OUTCOMES

1. Dobrzanski, M.J., Reome, J. B., Gilmartin, L.M. and Dutton, R.W. 2002. Type 1 and Type 2 Effector Cell Subpopulations In Progressive Breast Cancer. (*Abst*) Era of Hope 2002 Department of Defense Breast Cancer Research Program Meeting-Orlando, FL.
2. HA-Transfected TS/A Mammary Adenocarcinoma Cell Line (TS/A-HA).

CONCLUSIONS

These studies performed in year one of this proposal includes the development and characterization of a murine breast cancer model that correlates, in part, with stages of clinically progressive disease. We have also characterized the endogenous T cell immune responses in mice at various stages of tumor growth and maturation. Although we show that "activated" effector TIL cell populations (predominantly CD4 with fewer CD8 T cells) are present in the tumor with different kinetics and with various functional phenotypes, their responses appear none-the-less ineffective in preventing tumor growth and metastases. We have also shown that tumor-bearing mice produce substantial amounts of select chemokines (IP-10) and immunoregulatory cytokines (MIP, and TGF- β) that may, in part, affect immune cell function at the sites of tumor growth. Collectively, we suggest that the differential recruitment and presence of select immune cells and/or immunoregulatory factors (i.e. chemokines/cytokines) within the tumor environment at select stages of tumor maturation may aid, in part, in regulating effective host antitumor responses that result in aggressive malignancy. In our parallel studies using adoptively transferred tumor-reactive Tc1 and Tc2 CD8 effector T cell subpopulations as potential therapeutic agents, we can not only determine if these cells are therapeutically effective in tumor regression but also investigate their potential affects on what appears to be a "relatively ineffective" endogenous immune response in mice with progressive disease. The results of these studies will aid in understanding the underlying mechanisms of the efferent phase of the antitumor T cell response and provide insight for development of more effective therapeutics to breast cancer.

REFERENCES: None

APPENDICES: None